

Appl. No. 10/079,134
Am. Dated October 7, 2003
Reply to Office Action of August 8 2003

Amendment to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (currently amended) A method of identifying a nucleotide in at least a first position in a polynucleotide sequence, comprising:

providing a polynucleotide target sequence;

hybridizing the target sequence with a first oligonucleotide probe, wherein:

the probe comprises a first subsequence of nucleotides, a first 3'-terminal nucleotide, and a first fluorescent label coupled to the 3'-terminal nucleotide;

the subsequence is complementary to a portion of the target sequence that is immediately adjacent to the first position; and

the 3'-terminal nucleotide is complementary to one possible nucleotide in the first position;

contacting the hybridized probe and target sequence with polymerase extension reagents in a first extension reaction mixture;

~~monitoring/measuring a level of polarized fluorescence emitted fluorescent signal~~ from the first extension reaction mixture, ~~a decrease in polarized fluorescence~~ that is indicating ~~the presence or absence of polymerase extension of the probe, the presence of~~ polymerase extension of the probe indicating that the 3'-terminal nucleotide is complementary to the nucleotide in the first position; and

identifying the nucleotide in the first position.

2-3. (cancelled).

4. (currently amended) The method of claim 1, wherein the polymerase extension reagents include a 5'-3' 5'-DNA polymerase enzyme.

5. (cancelled).

6. (original) The method of claim 1, wherein the probe is from about 10 to about 50 nucleotides in length.

Appl. No. 10/079,134
Am. Dated October 7, 2003
Reply to Office Action of August 8 2003

7. (original) The method of claim 1, wherein the subsequence is from about 9 to about 49 nucleotides in length.

8. (original) The method of claim 1, wherein the polymerase extension reagents comprise a non-proofreading polymerase.

9. (currently amended) The method of claim 8, wherein the non-proofreading polymerase is selected from exonuclease exonuclease-minus klenow fragment, Taq polymerase, and Thermosequenase~~Thermosequenase~~.

10. (original) The method of claim 1, wherein the contacting step occurs in a channel of a microfluidic device.

11. (cancelled)

12. (currently amended) The method of claim 1, further comprising: hybridizing the target sequence with a second oligonucleotide probe that comprises:

the first subsequence of nucleotides, a second 3'-terminal nucleotide, and a second fluorescent label coupled to the second 3'-terminal nucleotide, the second fluorescent label being distinguishable from the first fluorescent label; and

the second 3'-terminal nucleotide is different from the first 3'-terminal nucleotide and is complementary to one possible nucleotide in the first position; and

wherein the monitoring measuring step comprises monitoring measuring a level of polarized fluorescence emitted fluorescent signals from each of the first and second fluorescent labels, a decrease in the amount of polarized fluorescence the fluorescent signal from one of the first and second fluorescent labels being indicative of polymerase extension of the first or second oligonucleotide probe, respectively.

13. (currently amended) A method for identifying a nucleotide in a first position in a target nucleic acid sequence, comprising:

amplifying the target nucleic acid sequence in a first reaction mixture that includes effective amounts of polymerase enzyme and four dNTPs;

introducing into the first reaction mixture a first primer sequence to produce a second reaction mixture under conditions conducive to a polymerase mediated primer extension,

Appl. No. 10/079,134
Am. Dated October 7, 2003
Reply to Office Action of August 8 2003

wherein the first primer sequence comprises a first subsequence of nucleotides, a first 3'-terminal nucleotide, and a first fluorescent label coupled to the 3'-terminal nucleotide, wherein the subsequence is complementary to a portion of the target sequence that is immediately adjacent to the first position, and the first 3'-terminal nucleotide is complementary to one possible nucleotide in the first position;

monitoring/measuring a level of polarized fluorescence emitted fluorescent signal from the second reaction mixture, a decrease in the amount of polarized fluorescence being that is indicative of the presence or absence of extension of the first primer sequence, the presence of polymerase extension of the first primer sequence indicating that the 3'-terminal nucleotide is complementary to the nucleotide in the first position; and

identifying the nucleotide in the first position based upon whether the fluorescent signal is indicative of the presence of extension of the first primer sequence.

14-18. (withdrawn).